Pakistan

Area planted to MYMV resistant mungbean and future needs in Pakistan

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Abstract

In Pakistan, mungbean is grown during *Kharif* (July to October) and summer (March to June) seasons. The popular mungbean varieties are NM 92 and NM 98. The mungbean area, production, and per acre yield has increased in the last few years due to the adoption of high yielding mungbean varieties, which increased the production of the farmers. The mungbean genetic improvement program at NIFA, Peshawar is concentrating both on the mutation breeding and incorporating desired traits through hybridization in the locally adapted material. Advanced lines of mungbean derived through hybridization of local and exotic germplasm have been evaluated at NIFA for some traits and screened for Mungbean Yellow Mosaic Virus (MYMV) during *Kharif* 2002 and 2003. Twelve recombinants gave significantly higher seed yield than the standard checks NM 92 and NM 98. The genotypes NFM-8-22 and NFM-12-9 had large seed size and genotypes NFM-6-2 and NFM-7-3 were early maturing. All the genotypes were resistant or highly-resistant to MYMV. The results of yield trials conducted in different locations as mono crop and as intercrop in plum orchards on farmers' field in *Kharif* 2003 are presented in this paper.

Introduction

Pulses are the major source of high quality protein in Pakistan. Chickpea (*Cicerarietinum* L.) and mungbean (*Vigna radiata* (L.) Wilczek) are the major *Rabi* (October to May) and *Kharif* seasons pulse crops respectively. Mungbean is also cultivated in the summer season. Cotton, rice, and maize are the major competitive crops with mungbean in irrigated areas, while millet and sorghum compete with mungbean in dry areas. Millet and sorghum are mostly grown for fodder purposes in the dry areas of Pakistan. In 2001, mungbean cultivated area was 239,200 ha, production was 115,400 t, and per acre yield was 482 kg ha^{-1} (Government of Pakistan, 2003). Mungbean cultivated area, production, and per acre yield has increased over the years (Table 1) but there is still a huge gap between experimental and national average yield. A large part of the cultivated area for mungbean is almost fixed, as no other crop is as economical as mungbean, but the area could be enlarged by planting mungbean as an intercrop or planting it on available fallow lands after wheat harvest in April/May (Khattak *et al.*, 2002ab). Since the temperature in May and June is high, it is essential to develop mungbean cultivars with short growth duration, high yield, and tolerance to high temperature.

In Pakistan, two-mungbean varieties namely NM 92 and NM 98 are presently cultivated on the major mungbean growing areas. Less than 10% of the mungbean-growing area is planted with land races or varieties released earlier. The mungbean seed industry in the country is not very progressive. The Punjab Seed Corporation is the only institute that supplies pure, quality mungbean seed to the farmers. Private sector seed companies are mainly engaged in supplying seeds of major and cash crops, e.g. cotton, wheat, rice, maize, and some vegetable seeds. The minor crops seed business involves more risk due to uncertain farmer demand for quality seed. Most of the farmers keep their own seeds from previous crop for future sowing. Pulse growers are mainly interested in procuring the seed of the newly released variety to get higher yield. Once the farmers obtain seeds of a newly released variety, they keep their own seeds for future planting.

The presently cultivated varieties in Pakistan are tolerant or resistant to MYMV. Studies on MYMV and screening of breeding material against this disease are a constant need. In hybridization programs for the improvement of yield potential of local varieties, improved lines from AVRDC are often used, but they are susceptible to MYMV. Resistance to MYMV is monogenic recessive but its degree of resistance depends on the number of modifier genes, which needs to be accumulated in a genotype to reduce the losses due to MYMV (Khattak *et al.*, 1999c, 2000). The development and performance of these advanced lines have been described in this paper.

Materials and methods

The exotic mungbean genotypes from AVRDC and India VC 3726, VC 1973A, VC 1560D, VC 1482C and Pusa Baisakhi were hybridized with local genotypes NM 92, NM 93, Blackmung, and 6601 (Table 2) in various cross combinations at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad during the

Table 1. Area, production and average yield (kg ha^{-1}) of mungbean in Pakistan, 1992-2002

Year	Area (000 ha)	Production (000 tons)	Yield (kg ha^{-1})
1992	146.8	62.1	423
1993	167.9	69.3	413
1994	179.7	80.0	445
1995	199.1	90.6	455
1996	192.4	89.5	465
1997	195.4	88.9	455
1998	199.5	90.5	453
1999	202.7	94.8	468
2000	219.2	104.5	477
2001	239.2	115.4	482

Table 2. Origin, hypocotyl color, plant pubescence, seedcoat color and reaction to MYMV of the parents used in hybridization

S. No.	Genotype/ Accession	Origin	Hypocotyl color	Plant pubescence	Seedcoat color	*MYMV reaction
1	VC 1482C	AVRDC	Green	Present	Shiny green	HS
2	VC 1560D	AVRDC	Green	Absent	Shiny green	HS
3	VC 1973A	AVRDC	Green	Present	Shiny green	HS
4	VC 3726	AVRDC	Green	Present	Shiny green	HS
5	Pusa Baisakhi	India	Purple	Present	Dull green	S
6	6601	Pakistan	Purple	Present	Shiny green	T
7	NM 36	Pakistan	Purple	Present	Shiny green	T
8	NM 93	Pakistan	Purple	Present	Shiny green	R
9	Blackmung	Pakistan	Purple	Present	Black	R
10	NM 92	Pakistan	Purple	Present	Shiny green	HR

*Reaction to MYMV where R is resistant, MR is moderately resistant, HR is highly resistant, T is tolerant.

Kharif season in 1998, following the crossing technique of Khattak *et al.* (1998) to develop high yielding genotypes for the *Kharif* season. The F_1 generation of these crosses was planted in summer 1999 and the hybrid plants were harvested individually. In *Kharif* 1999, the F_2 populations of each cross were raised as plant progeny rows for the selection of high yielding recombinants with resistance to MYMV . Mung Kabuli, a highly susceptible check for MYMV, was used as spreader and planted after each five rows to intensify MYMV inoculum. Chemical spray was avoided to maintain the natural population of whitefly (*Bemisia tabaci*) in the experimental field. The high-yielding and MYMV-resistant plants were chosen. Selected recombinants were advanced to confirm their breeding behaviour/ genetic stability for the desired traits. These were also screened for MYMV in subsequent generations.

The selected true breeding advanced lines were evaluated for yield and important agronomic traits in replicated yield trials at Peshawar during *Kharif* 2002 and 2003. The trials were conducted using RCBD with three replications at 6 rows per treatment. Row length was maintained at 4 meter. The plant-to-plant and row-to-row space was maintained at 10 cm and 30 cm respectively. The data recorded were as follows:

Days to flowering : Period from sowing to the opening of at least one flower in 50% of the plants of a genotype (in a plot) in each replication

Days to maturity : Period from sowing to the maturity of 90% of the pods on all plants of a genotype (in a plot) in each replication

Plant height (cm) : Average height of the 10 plants per replication as recorded from base of the plant to the top peduncle on the main branch

1000 seed weight (g): Weight of three random samples of 1000 seeds from a genotype per replication

Seed yield kg ha^{-1} : Weight of the seeds in the middle 4 harvested rows of a genotype in each replication

The average values of the two years data (2002 and 2003) were used in the analysis of variance. The recorded data of the parameters were subjected to analysis of variance (Steel and Torrie, 1980). All the calculations were performed through the microcomputer program MSTAT-C (Michigan State University and Agricultural University of Norway).

The true breeding lines were also screened for MYMV with two replications. Each treatment covered 4 rows with 4-meter length per replication in *Kharif* 2002 and 2003. Mung Kabuli, a highly-susceptible check for MYMV, was used as spreader. Chemical spray was also avoided. The percentage of MYMV infection was recorded on individual plant basis four weeks after sowing, when 100% of the highly-susceptible check mung kabuli were completely infected with MYMV. The mean disease score of each entry was calculated using the formula (infection rate x frequency)/total number of plants. The percentage of MYMV infection was converted to disease score and disease reaction, according to the MYMV disease score scale (0-8) reported by Malik (1992).

Evaluation on farmers field

The three advanced lines namely NFM-6-2, NFM-12-12, and NFM-13-2, and the two commercial varieties NM 92 and NM 98, were evaluated in replicated yield trials on farmers field during *Kharif* 2003. The Pakistan Science Foundation provided the funds for this purpose. Three replicated yield trials of the above-mentioned entries were planted at NIFA, Peshawar research farm and farmers field at D. I. Khan (major mungbean growing area) and Dir Bala (Highlands near Afghanistan border). Two non-replicated trials of the same entries were also planted in plum orchards at Peshawar. In the replicated trials, the five-mungbean varieties/ genotypes were planted using RCBD with three replications. Each variety/line was planted on a 7.2 m^2 plot with 6 rows at 4-meter length. The row-to-row and plant-to-plant space was maintained at 30 cm and 10 cm respectively. In non-replicated trials, the plot size was kept at 6 rows, each measuring15 meters long . The space between and within rows was the same as mentioned in replicated trials. The fertilizer was applied at the rate of N (25 kg) and P_2O_5 (60 kg) per hectare, or one bag of Diammonium phosphate (DAP) per acre, at the time of sowing. The crop was properly maintained by providing normal cultural practices. Manual weeding was done to remove weeds from the crop. In evaluating the extent of the MYMV disease, each trial planted in each location was visited at the flowering stage, with each entry examined visually. The data recorded were days to 50% flowering, days to maturity, plant height, 1000 seed weight, and seed yield. From the replicated yield trials, data were analyzed through analysis of variance using the computer program MSTAT-C, as mentioned earlier.

Results and Discussion

The mungbean advanced lines derived from eight different cross combinations have shown highly significant differences for seed yield (Table 3-5). The days to flowering and physiological maturity ranged from 36 to 49 days and 70 to 88 days, respectively. The plant height ranged from 58 to 99 cm while the 1000 seed weight from 38 to 56g. The reaction to MYMV was resistant to highly-resistant. The recombinants NFM-6-2, NFM-7-6, NFM-12-3, NFM-12-9, NFM-12-10, NFM-12-11, NFM-12-12, NFM-12-14, NFM-12-15, NFM-13-1, NFM-13-2, and NFM-13-4 have shown significantly higher seed yield than the checks NM 92 and NM 98. The genotypes NFM-8-22 and NFM-12-9 had the large seed size whereas the genotypes NFM-6-2 and NFM-7-3 took minimum days to maturity. The above genotypes could be used in hybridization program or in mutation breeding because of their distinctive traits such as high yield and early maturity, which are suitable in filling various gaps in the intensive cropping system for the *Kharif* season.

Table 3. Performance of mungbean advanced lines in yield trials conducted during *Kharif* 2002 and 2003 at NIFA, Peshawar

Entry	Parentage	Days to flowering	Days to maturity	Plant height (cm)	1000 seed wt (g)	*Reaction to MYMV	Yield (kg ha^{-1})
NFM-3-3	VC 3726 x NM 36	37	71	73	51	R	1334
NFM-6-1	VC 1971A x NM 92	37	76	66	55	MR	1403
NFM-6-2	VC 1971A x NM 92	36	70	67	52	R	1954
NFM-6-5	VC 1971A x NM 92	35	71	64	53	R	1330
NFM-7-3	VC 1560D x NM 92	36	70	62	54	R	1280
NFM-7-6	VC 1560D x NM 92	36	71	64	51	R	2019
NFM-7-13	VC 1560D x NM 92	39	71	67	50	R	1365
NFM-8-1	NM 93 x NM 92	37	72	64	55	R	1352
NFM-8-2	NM 93 x NM 92	39	71	64	54	R	1307
NFM-8-22	NM 93 x NM 92	41	71	70	56	R	1463
NFM-11-3	NM 92 x Blackmung	41	74	61	49	R	1375
NFM-11-4	NM 92 x Blackmung	42	75	60	46	R	1110
NM 92 (Check)	VC 2768B x NM 36	41	74	67	51	HR	1407
NM 98 (Check	NM 20-21 x VC 1482E	44	84	77	39	R	1491
SE		2.62	1.31	1.99	1.21	-	26.36
LSD 5% level		2.01	1.13	3.16	1.27	-	130.73

*Reaction to MYMV where R is resistant, MR is moderately resistant, HR is highly resistant.

Table 4. Performance of mungbean advanced lines in yield trials conducted during *Kharif* 2002 and 2003 at NIFA, Peshawar

Entry	Parentage	Days to flower-ing	Days to maturity	Plant height (cm)	1000 seed wt. (g)	*Reac-tion to MYMV	Yield (kg ha^{-1})
NFM-12-3	VC 1482C x NM 92	43	76	79	50	R	1956
NFM-12-5	VC 1482C x NM 92	42	76	70	52	R	965
NFM-12-6	VC 1482C x NM 92	42	74	65	52	R	1140
NFM-12-7	VC 1482C x NM 92	42	75	75	51	MR	1327
NFM-12-8	VC 1482C x NM 92	43	74	64	46	R	1358
NFM-12-9	VC 1482C x NM 92	42	79	89	56	R	1705
NFM-12-10	VC 1482C x NM 92	46	86	99	48	MR	1744
NFM-12-11	VC 1482C x NM 92	49	87	95	46	R	1806
NFM-12-12	VC 1482C x NM 92	43	77	82	52	R	1960
NFM-12-13	VC 1482C x NM 92	42	77	82	48	R	1320
NFM-12-14	VC 1482C x NM 92	47	82	85	51	R	1873
NFM-12-15	VC 1482C x NM 92	44	73	58	47	R	1886
NFM-12-16	VC 1482C x NM 92	46	73	60	52	R	1333
NFM-12-17	VC 1482C x NM 92	47	75	70	50	R	1252
NFM-12-18	VC 1482C x NM 92	41	73	60	54	R	1288
NM 92 (Check)	VC 2768B x NM 36	42	74	65	51	HR	1379
NM 98 (Check)	NM 20-21 x VC 1482E	45	86	80	39	R	1479
SE		1.56	3.16	3.10	0.85	-	31.06
LSD 5% level		1.29	1.56	4.13	0.0.61	-	108.92

*Reaction to MYMV where R is resistant, MR is moderately resistant, HR is highly resistant.

Table 5. Performance of mungbean advanced lines in yield trials conducted during *Kharif* 2002 and 2003 at NIFA, Peshawar

Entry	Parentage	Days to flowering	Days to maturity	Plant height (cm)	1000 seed weight (g)	*Reaction to MYMV	Yield (kg ha^{-1})
NFM-13-1	6601 x NM 92	42	78	73	49	MR	1412
NFM-13-2	6601 x NM 92	43	81	90	48	R	1830
NFM-13-4	6601 x NM 92	47	87	89	46	R	1540
NFM-13-8	6601 x NM 92	48	86	96	38	R	1137
NFM-14-3	NM 92 x Pusa Baisakhi	42	81	74	45	MR	1288
NFM-14-5	NM 92 x Pusa Baisakhi	43	78	69	54	R	1085
NFM-14-6	NM 92 x Pusa Baisakhi	42	78	75	45	R	1301
NFM-14-7	NM 92 x Pusa Baisakhi	43	78	72	46	R	1175
NFM-14-12	NM 92 x Pusa Baisakhi	43	81	79	52	R	1226
NM 92 (Check)	VC 2768B x NM 36	41	73	70	54	HR	1223
NM 98 (Check)	NM 20-21 x VC 1482E	47	88	83	38	R	1197
SE		2.47	3.93	4.67	0.89	-	21
$LSD_{(0.05)}$		1.66	2.69	3.27	0.46	-	91.62

*MYMV disease score

Plant parts infected/disease (%)	Score	Disease reaction
No infection	0	Immune (I)
1-5	1	Highly resistant (HR)
6-10	2	Resistant (R)
11-20	3	Moderately resistant (MR)
21-30	4	Tolerant (T)
31-40	5	Moderately tolerant (MT)
41-50	6	Moderately susceptible (MS)
51-80	7	Susceptible (S)
81-100	8	Highly susceptible (HS)

The mungbean genotypes with improved synchrony in pod maturity and determinate growth habit need to be selected and recommended for intercropping in sugar cane (Khattak *et al.*, 2002a,b). The selection of such traits would be beneficial in latter generations due to the predominant additive nature of inheritance of the synchrony in pod maturity and determinate growth habit in mungbean (Khattak *et al.,* 2001a, b, c, d). Seed size is the least sensitive trait for environmental fluctuations, in addition to the main yield-contributing factor in mungbean (Khattak *et al.*, 1995, 1999a, b). The high-yielding genotypes with large seed size are more preferred by the growers and consumers compared with genotypes having small seed size. The number of pods per plant is another main contributing factor toward yield but is very sensitive to environmental fluctuations (Khattak *et al.*, 2001e).

Evaluation on farmers field

In replicated yield trials, which were conducted as mono crop at NIFA (Table 6), Dir Bala (Table 7), and D. I. Khan (Table 8), the range for days to 50% flowering was 39-51 days; for days to 90% pods maturity, 68-87 days;1000 seed weight, 39-54 gram; and plant height from 68-100 cm. The genotypes showed significant genetic variability for seed yield in these trials. The genotype NFM-6-2 at NIFA, NFM-12-12 at Dir Bala, and NFM-6-2 at D. I. Khan produced the highest seed yields. The uniform maturity and lodging are desirable for monocropping in *Kharif* season. Both these traits are highly influenced by the environment and, as such, cannot be absolutely achieved.

Table 6. Performance of mungbean genotypes planted at NIFA, Peshawar research farm during *Kharif* 2003

Entry	Days to 50% flowering	Days to 90% pods maturity	Plant height (cm)	1000 seed weight (g)	Yield (kg ha^{-1})
NFM-12-12	43	79	90	51	1974
NFM-13-2	43	84	94	48	1702
NFM-6-2	40	74	70	52	2180
NM 92	40	76	82	52	1235
NM 98	42	87	100	40	1489
SE	0.53	0.70	0.89	0.87	12.9
$LSD_{(0.05)}$	1.74	2.28	2.9	0.52	42.08

Table 7. Performance of mungbean genotypes planted on farmer's field at Dir Balah during *Kharif* 2003

Entry	Days to 50% flowering	Days to 90% pods maturity	Plant height (cm)	1000 seed weight (g)	Yield (kg ha^{-1})
NFM-12-12	44	80	88	52	2105
NFM-13-2	43	79	89	49	1838
NFM-6-2	39	68	68	50	1694
NM 92	41	73	78	54	789
NM 98	43	81	89	40	1205
SE	0.83	1.2	076	0.92	30.87
$LSD_{(0.05)}$	2.71	3.90	2.47	0.48	100.79

Table 8. Performance of mungbean genotypes planted on farmer's field at D. I. Khan during *Kharif* 2003

Entry	Days to 50% flowering	Days to 90% pods maturity	Plant height (cm)	1000 seed weight (g)	Yield (kg ha^{-1})
NFM-12-12	48	82	89	52	1263
NFM-13-2	51	83	93	48	1072
NFM-6-2	49	80	70	49	1317
NM 92	47	78	83	53	812
NM 98	48	79	99	39	828
SE	0.66	076	0.89	0.85	47.88
$LSD_{(0.05)}$	2.16	2.49	2.91	0.37	156.16

In non-replicated yield trials conducted as intercrop in plum orchards (one was 7 years old and the other was 14 years old) at two different locations in Peshawar Valley showed completely different results than mono crop trials (Tables 9 & 10). The genotypes showed very low seed yield. All the entries had more days to flowering and maturity. The genotypes were taller in the 14-year old orchards compared with the 7-year old orchards. The reason was sunlight penetration, which the young orchards received more compared with the old ones. The low seed yield was common in both plum orchards. The main reason for the low seed yield in both orchards was the shading effect on mungbean crop. Therefore, mungbean is not a suitable crop for intercropping in the orchards in Peshawar valley.

Table 9. Performance of mungbean genotypes planted in the 14 year old Plum orchard on farmer's field at Peshawar during *Kharif* 2003

Entry	Days to 50% flowering	Days to 90% pods maturity	Plant height (cm)	1000 seed weight (g)	Yield (kg ha^{-1})
NFM-12-12	61	105	65	50	150
NFM-13-2	58	98	41	49	120
NFM-6-2	52	86	35	50	145
NM 92	58	87	38	52	95
NM 98	62	106	75	40	86

Table 10. Performance of mungbean genotypes planted in the 7 year old Plum orchard on farmer's field at Peshawar during *Kharif* 2003

Entry	Days to 50% flowering	Days to 90% pods maturity	Plant height (cm)	1000 seed weight (g)	Yield (kg ha^{-1})
NFM-12-12	54	95	85	52	205
NFM-13-2	53	96	84	48	194
NFM-6-2	49	84	65	49	215
NM 92	52	89	86	52	153
NM 98	59	98	93	38	164

The big difference between experimental and national yields in mungbean is in the management/cultural practices of the farmers. The mungbean area and total production could be increased by growing mungbean as an intercrop and growing it on fallow lands after wheat harvest. But to have increased yield, there is a need to develop high-yielding mungbean genotypes for the main *Kharif* season. These genotypes should be less sensitive to heavy rains and drought conditions. The uncertain heavy rains at the early stages cause death of the seedlings. Meanwhile, heavy rains at the flowering stage cause shedding of the flowers and promote vegetative growth.

The drought, no doubt, causes drastic reduction in mungbean yield. Therefore, genotypes tolerant to drought will surely increase the mungbean production in the country. Presently, the mungbean growers in the country need genotypes which could produce high and stable yield both under rainy and drought conditions during *Kharif* season.

Future needs

In addition to the development of high-yielding and disease-resistant mungbean genotypes, the following research areas are important to increase mungbean production in the country:

1. Improved synchrony in pod maturity and low vegetative growth after the initiation of reproductive phase (comparatively determinate growth habit).
2. Higher seed yield in the first flower flush.
3. Tolerant to moisture stress.
4. Low vegetative growth response during prolonged cloudy/rainy weather.
5. Tolerant to flower/pod shedding effects.
6. Tolerant to bruchid attack/infestation.

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Mungbean production in the North West Frontier Province of Pakistan and the influence of on-farm seed priming

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Abstract

The effect of 'on-farm' seed priming, or soaking seeds in water for eight hours before surface-drying and sowing them, was tested for mungbean (*Vigna radiata* (L.) Wilczek) in 15 irrigated on-station trials and four sets of rainfed, paired-plot, farmer-participatory trials over four years from 1999 to 2002 in the North West Frontier Province of Pakistan. Of the 19 trials, priming was significantly better than non-priming in 14 trials with a mean yield increase of 53%. In the remaining five trials there was no significant difference between treatments but in no case was priming worse than not priming. In a subset of 11 on-station trials in which management was considered to be optimal, yield declined in a linear fashion as the date of sowing was delayed. The rate of decline of about 30 kg ha^{-1} d^{-1} after June 1 was similar for both non-primed and primed crops, although the latter declined from a higher base. An additional trial in 2002 investigated the effect of priming on the incidence and severity of infection of mungbean with Mungbean Yellow Mosaic Virus (MYMV). More than 70% of the non-primed plants were observed to have severe or lethal symptoms whereas only 14% of the primed plants were similarly affected. Only 9% of non-primed plants showed no disease symptoms in contrast to 32% of primed plants. The primed crop produced 80% more above ground biomass (3.3 t ha^{-1} versus 1.9 t ha^{-1}), 264% more pod yield (1.0 t ha^{-1} versus 0.28 t ha^{-1}), and 415% more grain (0.36 t ha^{-1} versus 0.07 t ha^{-1}) than did the non-primed crop. Farmers' yields were proportional to rainfall over the four years and the mean increase in grain yield due to priming in the 39 farmers' trials was 33%. It was concluded that 'on-farm' seed priming is a low-cost, low-risk technology that has the potential to raise mungbean yields substantially, thus making it a more attractive crop for farmers.

Introduction

In Pakistan, mungbean is grown on an area of 219,200 hectares with production of 104,500 tons under rainfed and irrigated conditions. In the North West Frontier Province, the area under mungbean cultivation is 9,500 ha with production of 6,100 tons. In the province, half of the mungbean area is irrigated while the other half is dependent on rains (Government of Pakistan, 2002). Yields for the rainfed area are generally low and variable due to sparse, erratic rainfall and marginal soils. In Punjab province, mungbean production is dependent mainly on surface irrigation but it is also grown under rainfed conditions. In the southern region of Pakistan rainfall is scanty and mungbean is grown with surface irrigation only.

Poor crop establishment is a major constraint for mungbean production (e.g., Naseem *et. al.*, 1997; Kirchhof *et al.*, 2000; Rahmianna *et al.*, 2000) and high yields have been correlated with early vigour (Kumar *et al.*, 1989). A simple, low-cost, low-risk technology called 'on-farm' seed priming has been shown to improve emergence, seedling vigour and yield in a range of crops, including legumes (Harris *et al.*, 1999; 2001; 2002a, b; Kumar *et al.*, 2002; Musa *et al.*, 2001). On-farm seed priming, or soaking seeds in water before sowing, has also been reported to increase resistance to disease in chickpea (Musa *et al.*, 2001). Mungbean Yellow Mosaic Disease (MYMV) is a serious disease of mungbean and other legumes in Pakistan (Ayub and Ilyas, 1988; Bashir *et. al.*, 1991), elsewhere in South Asia (Kaiser *et. al.*, 1968) and worldwide (Bashir *et. al.*, 2000). The disease is primarily transmitted by the whitefly *Bemisia tabaci* Gemm., although Bashir *et al.* (2000) have reported a low incidence (around 3%) of seed-borne transmission.

This paper reports the results of on-station (irrigated) and participatory on-farm (rainfed) trials to determine the effectiveness of 'on-farm' seed priming in mungbean over four contrasting seasons between 1999 and 2002. The incidence and severity of infection with MYMV were also investigated, together with components of yield, in primed and non-primed mungbean fields during 2002.

Materials and methods

Experimental sites

All trials are summarised in Table 1. Mini-plot and research station trials were all implemented at the NWFP Agricultural University research station which has a warm to hot semi-arid sub-tropical continental climate with mean annual rainfall of about 360 mm. Soils are mostly fine textured, derived from piedmont alluvium, deep and well-developed, and belong to Great Group Haplustalfs. Soil in the area is a silty clay loam, well drained and strongly calcareous, with a pH of 8.2. The soil is deficient in nitrogen and phosphorus but has adequate potassium. Organic matter is less than 1%. Rainfall data were collected at this site and are summarised in Table 2.

Farmer-participatory trials were implemented in the Karak area which is approximately 200 km south of Peshawar. Mean annual rainfall here is less than 400 mm. Loamy sands and sandy loams were formed from weathering of surrounding sandstone rocks and deposited by rain torrents. Soil mostly belong to Great Groups, namely Torrifluvents and Torripsamments. Underground water is deep and saline. There are few sources of irrigation, and agriculture is mostly dependent on rainfall. Soils are non-saline, slightly to strongly calcareous (average lime content 10 %) with an alkaline reaction (pH around 8.2). Organic matter was less than 0.5%.

Experimental design and cultural details

Mini-plots were used for preliminary studies and investigations that required frequent measurements. Research station trials used larger plots and thus were more representative of field conditions. All trials at Peshawar received diammonium phosphate (DAP) fertilizer at the rate of 24 kg ha^{-1} N and 60 kg ha^{-1} P2O5. Pests and diseases were controlled and plots were irrigated as necessary. All mini-plot and research station trials were laid out as randomized complete block designs (except for one non-replicated, broadcast, demonstration trial, 01/3 in Table 1) and were sown by hand with known numbers of seeds per plot, using 0.05 x 0.5 m spacing for a nominal population density of 40 plants m^{-2}. The duration of priming varied between trials (Table 1) but the treatment always consisted of soaking seeds in fresh water for the required time, pouring off the water, surface-drying the seed to facilitate handling and sowing immediately. All trials used cv. NM 92, although additional varieties were tested in two trials in 1999 (see Table 1). Observations and measurements were made as noted in Table 1.

Table 1. Summary of experiments with seed priming in mungbean in North West Frontier Province, Pakistan, 1999-2002.

Site	Expt. No.	Sowing date	Priming (h)	Plot size (m)	Design & Replication	Harvest date	Observations (s = sequential picking)
1999							
Mini-plots	99/1	6 June	0, 8	1.5 x 2	†RCBD, 2	11 June	Emergence
Mini-plots	99/2	16 July	0, 8	1.5 x 2	RCBD, 4	3 Oct	Grain yield
Res. Station	99/3	25 June	0, 4, 8, 12	2.5 x 4	RCBD, 4	13 Sept	Emergence, yield components
Res. Station	99/4	20 July	0, 8	2.5 x 8	RCBD, 4	20 Oct	Grain yield
Res. Station***	99/5	6 Aug	0, 8	2.5 x 8	RCBD, 3	1 Nov	Emergence, grain yield
Res. Station	99/6	22 July	0, 8	2.5 x 6	RCBD, 4	10 Oct	Grain yield
Farmers' fields***	99/7	24-27 July	0, 8	30 x 30	Paired plots, 5	15-20 Oct	Grain yield
2000							
Mini-plots	00/1	19 June	0, 4, 8, 12	1.5 x 1	RCBD, 4	20 Sept	Yield components
Res. Station	00/2	12 June	0, 4, 6, 8	2.5 x 4	RCBD, 4	10 Sept	Yield components
Res. Station	00/3	25 June	0, 4, 6, 8	2.5 x 4	RCBD, 4	13 Sept	Yield components
Res. Station	00/4	7 July	0, 8	2.5 x 6	RCBD, 4	12 Oct	Yield components
Farmers' fields	00/5	14-17 July		30 x 30	Paired plots, 7	10-14 Oct	Grain yield
2001							
Mini-plots	01/1	17 July	0, 8	0.9 x 1.1	RCBD, 3	11/9, 26/9, 9/10, 18/10	Yield components (s)
Res. Station	01/2	30 June	0, 8	2.5 x 4	RCBD, 4	18 Sept	Emergence, yield components
Res. Station	01/3	1 July	0, 8	20 x 50	Demonstration, 1	25 Sept	Yield components
Farmers' fields	01/4	10-15 July	0, 8	30 x 30	Paired plots, 7	10-12 Oct	Grain yield
2002							
Mini-plots	02/1	6 June	0, 8	2.1 x 2.4	RCBD, 6	2/8, 19/8, 9/9, 17/9	Emergence, yield components (s)
Res. Station	02/2	15 June	0, 8	4 x 4	RCBD, 4	21/8, 10/9, 30/9	Emergence, yield components (s)
Res. Station	02/3	21 June	0, 8	4 x 5	RCBD, 4	23/8, 11/9, 23/9	Emergence, yield components (s)
Res. Station	02/4	28 June	0, 8	40 x 50	RCBD, 4	24 Sept	Emerg., MYMV symptoms, yield
Farmers' fields	02/5	17-20 July	0, 8	30 x 30	Paired plots, 20	12-17 Oct	Stand density, grain yield

Note: All trials used variety NM 92. Those trials marked *** tested additional varieties.
RCBD = Randomised Complete Block Design.

Paired-plot trials were implemented by five farmers in 1999, seven farmers in 2000, seven in 2001, and 20 in 2002. In 1999, all farmers used cv NM 92 plus a local variety; later, cv NM 92 was used alone in subsequent years. All farmers' trials were sown by broadcasting seed (soaked for 8 hours in a plot adjacent to a plot using the same amount of non-primed seed) at the rate of 40 kg ha^{-1} (weighed before soaking) then ploughing using bullock- or donkey-drawn ploughs. DAP was applied at a rate of 125 kg ha^{-1} (24 kg N, 60 kg P_2O_5) without irrigation. The farmers' normal weeding and other cultural practices were followed. Yields were assessed as the mean of four 1 m^2 quadrats placed randomly in each plot. All data were subjected to analysis of variance.

Disease incidence and severity (Trial 02/4 only, Table 1)

The number of plants and the proportion of those plants showing MYMV disease symptoms were measured during the final stage of pod formation (68 DAS) within four 4m x 4m randomly-sited quadrats in each of the eight plots. A visual scoring index (VSI), based on external symptoms such as stunted growth, chlorosis and flower malformation, was used as described by Bashir and Hassan (1998) and Hassan *et al.*, (2003). The severity of the symptoms shown by each plant in the quadrat was used to categorize plants into: 0 = no infection; 1 = mild; 2 = moderate; 3 = severe; 4 = lethal. No measurements were made on whitefly incidence.

Table 2. Monthly rainfall (mm) at Peshawar, NWFP, Pakistan 1998-2002

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
1998	40	140	61	86	28	13	81	46	50	18	0	0	562
1999	132	28	84	21	3	20	13	34	33	0	24	0	393
2000	66	24	3	3	10	3	84	68	57	12	0	12	342
2001	0	4	38	21	12	0	29	10	9	0	10	0	133
2002	3	45	12	15	18	96	107	65	74	12	2	18	468
30-y mean	39	41	65	42	40	7	39	41	14	10	10	15	363

Results

The effect of seed priming on grain yield in all trials is summarized in Table 3. Seed priming increased grain yield in all 19 (not including 02/4) trials spread over four contrasting years (Table 1), including both irrigated and rainfed environments, and a range of sowing dates. These increases were statistically significant in 14 out of these 19 trials with an average increase of 53% as a result of priming for eight hours. There were no trial results that showed priming was worse than not priming.

The results of trials on the duration of soaking (99/3, 00/1, 00/2, and 00/3 in Table 3) showed that the optimum time was 6 - 8 hours, and that soaking for 12 hours reduced the benefits. Consequently, seed was primed for eight hours in all other trials.

All trials used the popular cultivar NM 92. Two trials in 1999 (99/5 and 99/7) showed that other cultivars also responded positively to seed priming. Results (not shown) from 99/5 indicate no interaction between priming and cultivar. The 29% yield increase due to priming shown for this trial in Table 3 is the mean of all cultivars used, while the 27% increase in yield is from 99/7. All cultivars similarly responded positively to priming when tested by five farmers in Karak.

Table 3. Summary of yield responses to seed priming in mungbean in North West Frontier Province, Pakistan, 1999-2002

(Experiments are described in Table 1 and in the text.)

Site	Expt. No.	Priming (h)	Yield of non-P treatment (kg ha^{-1})	% increase due to priming	[2]Significance
1999					
Mini-plots	99/1	0, 8	Emergence only	Not appl.	Not appl.
Mini-plots	99/2	0, 8	1059	16	*
Res. Station	99/3	0, 4, 8, 12	1502	38, 60, 39	***
Res. Station	99/4	0, 8	336	206	**
Res. Station	99/5	0, 8	404	29	*
Res. Station	99/6	0, 8	777	58	**
Farmers' fields	99/7	0, 8	1056	27	***
2000					
Mini-plots	00/1	0, 4, 8, 12	1843	33, 96, 46	***
Res. Staion	00/2	0, 4, 6, 8	2562	12, 23, 52	***
Res. Station	00/3	0, 4, 6, 8	1500	39, 61, 39	***
Res. Station	00/4	0, 8	1560	44	NS
Farmers' fields	00/5	0, 8	741	43	***
2001					
Mini-plots	01/1	0, 8	653	52	***
Res. Station	01/2	0, 8	1840	6	NS
Res. Station	01/3	0, 8	1440	13	*
Farmers' fields	01/4	0, 8	381	39	NS
2002					
Mini-plots	02/1	0, 8	3419	26	***
Res. Station	02/2	0, 8	1859	13	NS
Res. Station	02/3	0, 8	1551	12	NS
[1]*Res. Station*	*02/4*	*0, 8*	*70*	*416*	**
Farmers' fields	02/5	0, 6	1100	28	***

[1]*Not included in overall analysis – see text*

[2]* significant at 0.05 level , ** significant at 0.01 level , *** significant at 0.001 level, NS not significant at 5% level.

The rate of emergence with time was measured in seven trials (99/1, 99/3, 99/5, 01/2, 02/1, 02/2, and 02/3 in Table 1). In all cases, seedlings from primed seeds emerged earlier and faster, although the final stand density was not always statistically different between the two treatments. An example (trial 02/1) is shown in Figure 1 where a much larger proportion of the primed seed had germinated and seedlings emerged 100 hours after sowing (HAS) than that of the non-primed seed. The trend continued after 100 HAS although differences between treatments were no longer significant. This emergence pattern was observed in all trials in which significant benefits from priming were obtained.

Components of yield at final harvest were measured in 11 trials (99/3, 99/5, 00/1, 00/2, 00/3, 00/4, 01/1, 01/2, 01/3, 02/1, 02/2, and 00/3 in Table 1). Typical examples are shown in Tables 4 and 5. Grain yield gains were associated with slight increases in stand density (although this was significant only in 00/2) and also with improved individual plant performance such as longer pods, more pods per plant, and grains per pod. Total dry matter, pod mass, and grain mass were all significantly increased by priming although harvest index was not significantly affected, which suggests that overall growth was stimulated. This is in contrast to mere partitioning to reproductive structures.

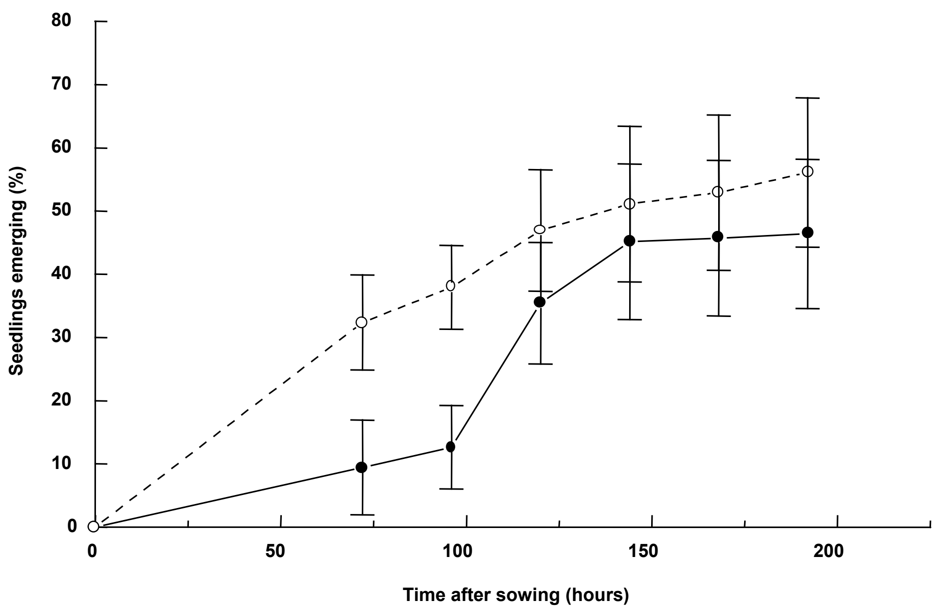

Figure 1. Emergence with time of non-primed seeds (closed symbols) and seeds primed for 8 hours (open symbols) in trial 02/1. Points are means of six replicates and bars are s.e.d. between means.

Table 4. Components of yield in experiment 99/3 (see Tables 1 and 3)

Variable	Priming treatment (hours)				Sig.[1]	LSD (5%)
	0	4	8	12		
No. of plants m^{-2}	24.7	25.5	27.0	26.2	NS	NS
Pod length (cm pod^{-1})	6.6	8.2	10.4	9.0	***	0.72
No. of pods $plant^{-1}$	12.5	14.0	19.0	14.8	**	3.1
No. of grains pod^{-1}	8.8	9.2	10.6	9.1	NS	NS
1000-grain weight (g)	45.5	57.9	65.3	59.6	NS	NS
Mass of shoots (kg ha^{-1})	2337	1894	3312	2875	NS	NS
Mass of pods (kg ha^{-1})	2304	3241	3896	3250	***	281
Total dry matter (kg ha^{-1})	4641	5135	7208	6125	**	1205
Mass of grains (kg ha^{-1})	1502	2083	2412	2094	***	114
Harvest Index	0.32	0.42	0.34	0.34	NS	NS

[1]* significant at 0.05 level , ** significant at 0.01 level , *** significant at 0.001 level, NS not significant at 5% level.

Table 5. Components of yield in experiment 00/2 (see Tables 1 and 3)

Variable	Priming treatment (hours)				Sig.[1]	LSD (5%)
	0	4	6	8		
No. of plants m^{-2}	28.7	30.0	31.5	30.0	*	1.69
Pod length (cm pod^{-1})	8.0	9.5	9.25	10.25	***	0.75
No. of pods $plant^{-1}$	18.0	17.0	16.3	17.5	NS	NS
No. of grains pod^{-1}	9.4	10.3	11.5	12.1	***	0.71
1000-grain weight (g)	50.0	54.9	55.1	56.6	**	3.1
Mass of shoots (kg ha^{-1})	1667	2291	2958	3542	***	391
Mass of pods (kg ha^{-1})	3480	3905	4274	5051	*	1083
Total dry matter (kg ha^{-1})	5146	6196	7233	8593	***	1016
Mass of grains (kg ha^{-1})	2562	2875	3146	3896	**	770
Harvest Index	0.50	0.47	0.44	0.46	NS	NS

[1]* significant at 0.05 level , ** significant at 0.01 level , *** significant at 0.001 level, NS not significant at 5% level.

The 11 research station trials were grown using recommended levels of inputs and should represent the potential of mungbean in Peshawar, yet yields varied widely from 336 kg ha^{-1} to 2,562 kg ha^{-1}. All trials were irrigated and were independent of rainfall, which varied yearly. However, the trials were sown over a wide span of time, which is from June 12 to August 6. The influence of sowing date on yield is shown in Figure 2. Yield of non-primed mungbean declined linearly with date of sowing. The yield of primed crops declined in an identical fashion – comparison of regression coefficients using a t-test showed no significant difference between treatments – albeit from a higher base. Early sowing is clearly important to produce a successful mungbean crop in NWFP.

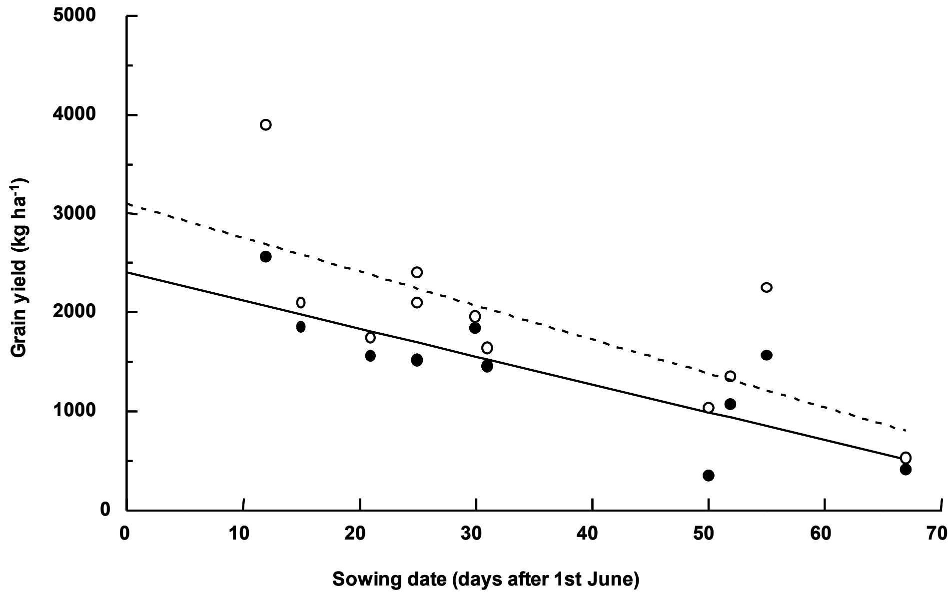

Figure 2. Effect of date of sowing (days after June 1) on grain yield of mungbean grown in 11 trials at the NWFPAU research station between 1999 and 2002. Non-primed yield (closed symbols, solid line) regression: y = 2400 – 28.1 x; R^2= 0.64. Primed yield (open symbols, broken line) regression: y = 3100 – 34.3 x; R^2 = 0.52. Both regressions are significant at P<0.05.

The low non-primed yield in 99/4 (Table 3) can be partly explained by the effect of late planting, but the very large (206%) response to priming may have had another cause. It was observed that this trial was particularly heavily affected by Mungbean Yellow Mosaic Virus (MYMV) disease, the incidence and severity of which were much less evident in the primed plots. The effect of MYMV was investigated in detail in trial 02/4 and the results are shown in Table 6.

Table 6. Plant population density m^{-2} at harvest (a), the percentage of health, moderately- or severely diseased plants based on a visual scoring index (VSI, 0-4) of symptoms of MYMV (b) and final yield components (c) of primed- and non-primed crops

Variable	Seed treatment		[a]Significance with SE diff.
	Not primed	Primed 8 hours	
a. Plant population density at harvest			
Total number of plants, m^{-2}	14.1	17.6	NS 2.0
[a]b. Disease incidence			
No disease (VSI=0), %	17.8(9.4)	33.7(31.6)	*4.49
Mild or moderate disease (VSI=1 or 2), %	25.4(18.7)	47.4(54.5)	** 2.74
Severe or lethal disease (VSI=3 or 4), %	58.3(72.0)	21.2(13.7)	** 4.97
c. Components of yield			
Total aboveground biomass, kg ha^{-1}	1859	3345	*355.9
Pod yield, kg ha^{-1}	284	1034	**127.9
Grain yield, kg ha^{-1}	70	361	**41.3

[a] Analysis of disease incidence used arcsin transformed data. Transformed means are presented (with non-transformed means in brackets). SE diff. values were calculated using transformed data.

The primed crop initially exceeded the target population density of 30–35 plants m^{-2} (based on an expected 60% field emergence observed in previous experiments – data not shown), whereas the non-primed treatment resulted in significantly fewer (32%) plants per unit area. Subsequent mortality throughout the crop cycle reduced the population density to below 20 plants m^{-2} by the time of final harvest, which is sub-optimal for mungbean in these conditions. Although the primed crop was slightly denser, the difference was not statistically significant (Table 6a).

The symptoms of MYMV infection were apparent on only 68% of the primed plants whereas 91% of non-primed plants showed symptoms of infection (Table 6b). Most (55%) of the primed plants showed only mild or moderate symptoms (VSI = 1 or 2), while most (72%) of the non-primed plants had severe or lethal symptoms (VSI = 3 or 4). There were statistically significant differences between the primed and non-primed treatments for each of the three VSI classes. The measurements clearly showed that the primed crop exhibited fewer observable symptoms of MYMV than the non-primed crop.

The primed crop, although not significantly denser than the non-primed crop (Table 6a), produced 80% more biomass, 264% more pod yield, and 415% more grain than the non-primed crop (Table 6c).

Rainfall in 1999 and 2000 was close to the long-term average, while in 2002 it was slightly above average, and in 2001, it was a very dry year (Table 1). This is reflected in the mean yields obtained in the farmers' rainfed trials where yield ranged from 381 kg ha^{-1} to 1100 kg ha^{-1} in direct proportion to the rainfall (99/7, 00/5, 01/4 and 02/5 in Table 3). Priming was associated with mean yield increase in farmers' trials in all four years, although the difference was not significant in 2001 (01/4 in Table 3). This is because of a very large variability among the seven trials though all responded positively to priming. Primed seed outperformed non-primed seed in 34 out of 39 trials, a 'success rate' for priming of 87%, and giving an overall 4-year mean yield advantage of 33%. Stand density, measured only in 2002 (02/5), was 37 plants m^{-2} in the non-primed crops and 43 plants m^{-2} in the primed plots, or an 18% increase ($P<0.01$). Results are discussed in more detail by Rashid *et al.* (2004a).

Discussion

This work has clearly demonstrated the effectiveness of 'on-farm' seed priming for mungbean grown in a wide range of contrasting conditions. These findings agree with positive responses to seed priming reported for other important tropical and sub-tropical crops such as maize in India, Zimbabwe, and Pakistan (Harris *et al.*, 1999; 2002a), chickpea in India and Bangladesh (Harris *et al.*, 1999; Musa *et al.*, 2001), wheat in India, Nepal and Pakistan (Harris *et al.*, 2001), upland rice in India (Harris *et al.*, 1999; 2002b), and finger millet in India (Kumar *et al*., 2002).

Without irrigation, grain yield of mungbean was clearly dependent on rainfall. Further, the yield of irrigated crops declined as sowing was delayed. This effect of time of sowing has been noted in Pakistan by Ramzan *et al.* (1992), and delayed sowing is associated with reduced flowering, pod setting, and maturity. These characteristics are caused by the temperature. Daily mean temperature declines sharply from 29 C in September to 18 C in November, and optimum mean temperatures for mungbean cultivation is in the range of 28-31 C (Malik, 1994). Moreover, disease is more serious in late sown crops (M.A.Khan, pers. comm.).

Yield has been correlated with the number of pods per plant (Pongkao and Yothasiri, 1995) while other authors have reported a rise in yield as a result of increased pod size and weight (Natarajan and Palanisamy, 1988). It is noteworthy that, in the work reported here, variables differed between trials with regard to their contribution on yield increase (compare the data in Tables 4 and 5).

Significant percentage increase in grain yield due to priming ranged from 16% to 206% (415% if 02/4 is included). However, analysis failed to show any correlation between these increases and the yield of the non-primed treatment. The effect of priming in mungbean seems to be consistently positive across the environments tested in this stiudy and yet independent of yield level.

Trial 02/4 clearly demonstrated that the growth and yield of primed mungbean was much less adversely affected by MYMV than the non-primed crop's. There was a five-fold increase in grain yield due to priming in this trial, as well as a marked increase in biomass (Table 6). The grain yield of the non-primed crop in this trial (70 kg ha^{-1}) was very low,as the average yield of mungbean in NWFP is around 600 kg ha^{-1} (Government of Pakistan, 2002) while that of the non-primed crops from the 11 irrigated on-station trials at the Peshawar site (Table 3) was 1375 kg ha^{-1}. The reason for the below-average yield in the 2002 trial are not known. However, biomass was quite large (Table 6) and it is possible that the relatively high rates of N applied (for a legume), together with a residual effect from N applied to the preceding wheat crop, might have stimulated vegetative growth at the expense of pods and grain.

There have been other reports that crops from primed seeds are more resistant to pests and diseases. Musa *et al.*, (2001) showed that primed chickpea crops had 37% fewer plants infected with collar rot (*Sclerotium rolfsii*) than crops from non-primed seeds, which contributed to a mean yield increase of 33% due to priming. Harris *et al.*, (1999) reported on Indian farmers' comments that primed chickpea was less damaged by pod borers. The authors were unable to confirm this effect in India. Pod borer damage on chickpea in other trials in Bangladesh was also much reduced in primed crops but the apparent difference was not statistically significant (Musa *et al.*, 2001).

The mechanism/reason behind seed priming's capability of increasing resistance to infestation and infection in mungbean and chickpea is unknown, but there are several possibilities. This resistance simply shows that plants have greater vigour following the more rapid emergence of primed seed (Figure 1). The faster development often associated with primed crops might reduce the size of infection "windows".

A third, more direct, mechanism is Systemic Acquired Resistance (SAR) that is analogous to the immune response in animals (e.g., Poonam Jindal Sohal and Bajaj, 1998), although seed priming has not yet been linked with this mechanism. These alternative mechanisms are discussed in more detail by Rashid *et al.* (2004b). Additional studies are underway to classify them.

In recent years, there has been a decline in mungbean yield per hectare in Pakistan, although total production has increased slightly because more area are now under cultivation (Government of Pakistan, 2002). The farmers' decision to grow mungbean might be influenced positively by the prospect of larger, perhaps more assured, yields associated with priming, as what happened in the case of chickpea in the Barind region of Bangladesh (Saha, 2002).

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